



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US97/21696 (22) International Filing Date: 20 November 1997 (20.11.97) (30) Priority Data: 60/031,194 20 November 1996 (20.11.96) US 60/035,266 12 December 1996 (12.12.96) US 60/053,200 21 July 1997 (21.07.97) US (71) Applicant (for all designated States except US): YISSUM RESEARCH DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM [IL/IL]; Jabotinsky Street 46, P.O. Box 4279, 91042 Jerusalem (IL). (71) Applicant (for MW only): KOHN, Kenneth, I. [US/US]; 6761 Alderly Way, West Bloomfield, MI 48322 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): SOREQ, Hermona [IL/IL]; HaMaayan Street_14, 95903 Jerusalem (IL). FRIEDMAN, Alon [IL/IL]; Moshav Maslul, 85112 M. Post HaNeguev (IL). SEIDMAN, Shlomo [IL/IL]; 90909 Neve Daniel (IL). KAUFER, Daniela [IL/IL]; Haguilboa Street_10/6, 90805 Mevasseret Zion (IL).		(74) Agents: KOHN, Kenneth, I. et al.; Kohn & Associates, Suite 410, 30500 Northwestern Highway, Farmington Hills, MI 48334 (US). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published With international search report. (88) Date of publication of the international search report: 17 September 1998 (17.09.98)	
(54) Title: A METHOD AND COMPOSITION FOR ENABLING PASSAGE THROUGH THE BLOOD-BRAIN BARRIER			
(57) Abstract <p>A pharmaceutical composition for facilitating passage of compounds through the blood-brain barrier comprising the agent ACHE-14 readthrough (SEQ ID No:1) splice variant or the I4 peptide (SEQ ID No:2) and analogues of each thereof and a pharmaceutically acceptable carrier is disclosed. Alternatively, the pharmaceutical composition for facilitating passage of compounds through the blood-brain barrier can comprise the agents adrenaline, atropine, dopamine and/or an adrenergic combination and a pharmaceutically acceptable carrier. The composition can comprise at least two of the agents. The composition of the present invention can optionally include the compound to be transported across the blood-brain barrier. Alternatively, the compound can be co-administered (simultaneously) with the composition or be administered at some point during the biologically effective period of the action of the composition. The present invention provides a method for administering a compound to the CNS of an animal by subjecting the animal to a stress-mimicking agent or treatment. This agent or treatment facilitates disruption of the blood-brain barrier. During the period that the BBB is opened or disrupted a compound can be administered such that the compound is enabled to pass through the disrupted BBB into the CNS.</p>			

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INTERNATIONAL SEARCH REPORT

Inten 1st Application No
PCT/US 97/21696

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/46 A61K31/135 A61K31/46

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 414 568 A (WASHINGTON UNIVERSITY) 27 February 1991 see the whole document	3-16
A	WO 95 23158 A (YISSUM RESEARCH DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSAL) 31 August 1995 see the whole document	1-17
A	EP 0 364 584 A (KI NII FARMAKOLOGII TOKSIKOLOG) 25 April 1990 see the whole document	1-17
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

15 May 1998

Date of mailing of the international search report

24.06.1998

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INTERNATIONAL SEARCH REPORT

Inter. nal Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>FRIEDMAN, ALON ET AL: "Pyridostigmine brain penetration under stress enhances neuronal excitability and induces early immediate transcriptional response"</p> <p>NAT. MED. (N. Y.) (1996), 2(12), 1382-1385, XP002065074</p> <p>see the whole document</p> <p style="text-align: center;">-----</p>	1-17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/21696

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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Although claim 17 is directed to a diagnostic method practised on the
2. ☐ Claims Nos.:
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page 1 of 2

INTERNATIONAL SEARCH REPORT

international application No.
PCT/US 97/21696

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page 2 of 2

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Although claims 6-16 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
Although claim 17 is directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/21696

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 414568 A	27-02-1991	US 4988710 A US 5011853 A	29-01-1991 30-04-1991
WO 9523158 A	31-08-1995	AU 2096395 A EP 0750627 A	11-09-1995 02-01-1997
EP 0364584 A	25-04-1990	WO 8910124 A	02-11-1989



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A METHOD AND COMPOSITION FOR ENABLING
PASSAGE THROUGH THE BLOOD-BRAIN-BARRIER

BACKGROUND OF THE INVENTION

5

1. FIELD OF THE INVENTION

The present invention relates to a method and composition for transporting compounds including pharmaceutical compositions across the Blood-Brain Barrier (BBB).

2. DESCRIPTION OF RELATED ART

The Blood-Brain Barrier (BBB) maintains a homeostatic environment in the central nervous system (CNS). The capillaries that supply the blood to the brain have tight junctions which block passage of most molecules through the capillary endothelial membranes. While the membranes do allow passage of lipid soluble materials, such as heroin and other psychoactive drugs, water soluble materials such as glucose, proteins and amino acids do not pass through the BBB. Mediated transport mechanisms exist to transport glucose and essential amino acids across the BBB. Active transport mechanisms remove molecules which become in excess, such as potassium, from the brain. For a general review see Goldstein and Betz, 1986 and Betz et al, 1994, incorporated herein in their entirety by reference.

The BBB was initially observed by Ehrlich when he observed what he termed "lower affinity" of vital dyes for the brain than other tissue. Goldmann in 1913 however, determined the actual presence of a barrier by showing that the vital dye trypan blue when injected directly into the brain stained the brain but did not

leave the CNS. These early experiments by Golmann and others established that the CNS is separated from the bloodstream by blood-brain and blood-cerebrospinal fluid (CSF) barriers.

5 The BBB impedes the delivery of drugs to the CNS. Methods have been designed to deliver needed drugs such as direct delivery within the CNS by intrathecal delivery can be used with, for example, an Ommaya reservoir. United States Patent 5,455,044 provides for use of a
10 dispersion system for CNS delivery or see United States Patent 5,558,852 for a discussion of other CNS delivery mechanisms as well as Betz et al [1994] and Goldstein and Betz [1986].

 There has been some progress in designing drugs that
15 utilize the structure and function of the BBB itself to deliver the drugs. These drugs are designed to be lipid soluble or to be "piggy-backed" into the CNS by being coupled to peptides that can cross the BBB through mediated transport mechanisms. However, not all drugs
20 are amenable to this solution. Partridge and his colleagues have worked extensively in this area. Pharmacological formulations that cross the blood-brain barrier can be administered. [Brem et al., 1993] Such formulations can take advantage of methods now available
25 to produce chimeric peptides in which the present invention is coupled to a brain transport vector allowing transportation of these engineered drugs across the barrier [Partridge, et al., 1992; Partridge, 1992; Bickel, et al., 1993]. See also The Economist, January
30 4, 1997.

 In the disease process, the BBB is often disrupted. For example in meningitis, Tuomanen [1993] has shown that the response against the bacterial infection lead to a breach of the BBB. Further, in trauma and brain tumors
35 the BBB is often disrupted as well as exposure to certain

agents such as soman [Lallement et al, 1991; Petralli et al, 1991]. Disruption has been shown in ischemia [Burst, 1991] and in Alzheimer's Disease [Harik and Kalaria, 1991].

5 In appropriate cases the blood-brain barrier disruption can be utilized to deliver drugs to the CNS, as for example osmotic disruption [Neuwelt et al., 1980a]. However, generally this is not the case since, for example, exposure to soman is accompanied by seizures
10 [Petralli et al, 1991].

 However, while these methods do provide CNS delivery for some drugs it would be useful to have additional means of delivery. In particular it would be useful to have mechanisms that temporarily and reversibly open the
15 BBB to allow non-engineered drugs through.

 Stress has been shown to affect the permeability of the BBB [Sharma, et al, 1991; Ben-Nathan, et al, 1991]. Further, in mammals, acute stress elicits a rapid, transient increase in released acetylcholine (ACh) with a
20 corresponding phase of increased neuronal excitability [Imperato, et al, 1991]. There have been some studies showing that the pharmacological blockade of acetylcholine - hydrolyzing enzyme, acetylcholine esterase (AChE) promotes a similar enhancement in
25 electrical activity in cortical neurons [Ennis and Shipley, 1992].

 AChE has three splice variant AChEmRNAs (Figure 1). Alternative splicing controls the generation of proteins with diverse properties from single genes through the
30 alternate excision of intronic sequences from the nuclear precursors of the relevant mRNAs (Pre-mRNA). It is known to be cell type-, tissue- and/or developmental stage-specific and is considered as the principal mechanism controlling the site(s) and timing of expression and the

properties of the resultant protein products from various genes.

Three alternative AChE-encoding mRNAs have been described in mammals (Figure 1). The dominant brain and muscle AChE (AChE-T) is encoded by an mRNA carrying exon E1 and the invariant coding exons E2, E3, and E4 spliced to alternative exon E6. AChEmRNA bearing exons E1-4 and alternative exon E5 encodes the glycolipid phosphatidylinositol (GPI)-linked form of AChE characteristic of vertebrate erythrocytes (AChE-H). An additional readthrough mRNA (AChE-I4) species (Table 1, SEQ ID No:1) retaining the intronic sequence I4 (SEQ ID No:2; Figure 2) located immediately 3' to exon E4 is found in rodent bone marrow and erythroleukemic cells and in various tumor cells lines of human origin. (The book Human Cholinesterases and Anticholinesterases by Soreq and Zakut (Academic Press, Inc., 1993) provides a summation of the biochemical and biological background as well as the molecular biology of human cholinesterase genes and the proteins. The book in its entirety is incorporated herein by reference.)

It would be useful to facilitate transport through the BBB by using a stress mimicking agent to have a controlled reversible disruption, or opening, of the BBB and/or blood-CSF.

SUMMARY OF THE INVENTION

According to the present invention, a pharmaceutical composition for facilitating passage of compounds through the blood-brain barrier comprising the AChE-I4 readthrough (SEQ ID No:1) splice variant or the I4 peptide (SEQ ID No:2) and analogues of each thereof and a pharmaceutically acceptable carrier is disclosed.

Alternatively, the pharmaceutical composition for facilitating passage of compounds through the blood-brain barrier can comprise adrenaline, atropine and dopamine and a combination of dopamine and propanolol and a pharmaceutically acceptable carrier. Combinations of these agents can also be used.

The composition of the present invention can optionally include the compound to be transported across the BBB. Alternatively, the compound can be co-administered (simultaneously) with the composition or can be administered at some point during the biologically effective period of the action of the composition. That is the composition facilitates disruption of the BBB, i.e. opens the BBB, for a period depending on the dose and the compound can be administered during this relevant period.

The present invention provides a method for administering a compound to the CNS of an animal by subjecting the animal to a stress-mimicking agent or treatment. This agent or treatment facilitates disruption of the blood-brain barrier. During the period that the BBB is opened or disrupted a compound can be administer such that the compound is enabled to passage through the disrupted BBB into the CNS.

The method and composition of the present invention therefore provides for delivery to the central nervous system of compounds that are necessary for treatment modalities in any condition affecting the central nervous system where the blood-brain barrier would impede the delivery of the compound. These conditions can include any disease or pathology of the central nervous system and can include neuropsychiatric disorders. The method and composition of the present invention is an improvement of currently available means of delivery of

compounds to the central nervous system through the blood-brain barrier.

DESCRIPTION OF THE DRAWINGS

5 Other advantages of the present invention will be readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings wherein:

10 FIGURE 1 is a schematic diagram of the three splice variants of AChE.

 FIGURE 2 is shows the amino acid sequences of human (H) AChE variants from the end of E4 to the end of the protein in the three variants, E1-4,6 (SEQ ID No:3), E1-5 (SEQ ID No:4), E1-4-I4-E5 (readthrough; SEQ ID No:5).

15 FIGURES 3A-B show that acute stress and anticholinesterases modulate CNS gene expression similarly. Figure 3A are photographs showing RT-PCR analysis, C-fos RT-PCR traces represent mRNA preparations from 10 minute post-treatment, ACHE, synaptophysin and ChAT products represent RNA preparations from 30 minute following either stress or AChE inhibition. One out of 6 reproducible in vivo and in vitro experiments is shown. Figure 3B are extracellular evoked potentials recordings of stratum oriens fibers using glass microelectrodes in the CA1 area before (Control) or 30 minutes following addition of 1 μ M DFP (Anti-AChE), to the perfusing solution. Note the increased amplitude and duration of evoked extracellular field potentials and the enhanced paired-pulse facilitation. One out of 5 experiments showing AChE inhibition promotes neuronal excitability.

25 FIGURES 4A-D show delayed suppression of the hyperexcitation evoked by AChE inhibition. Graphs at (A) One hour administration of 1 or 10 μ M carbachol, (B) average and standard deviation values for 6 measurements

30

35

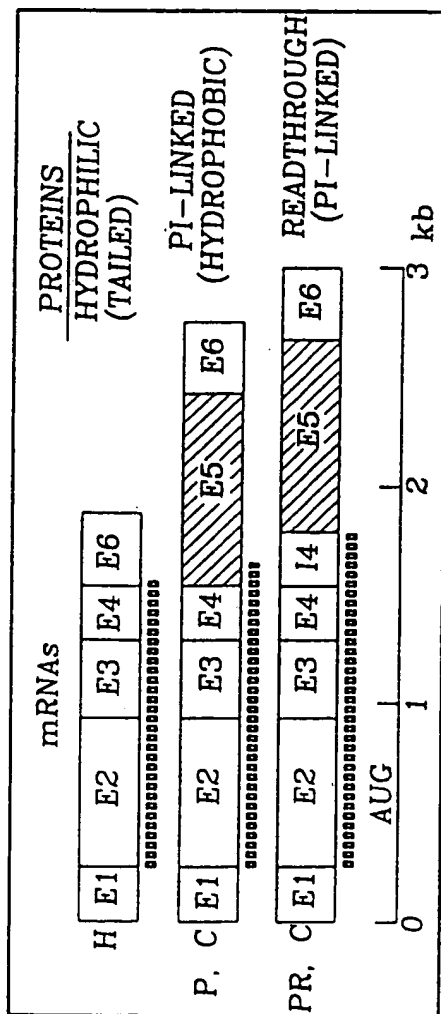


FIGURE 1

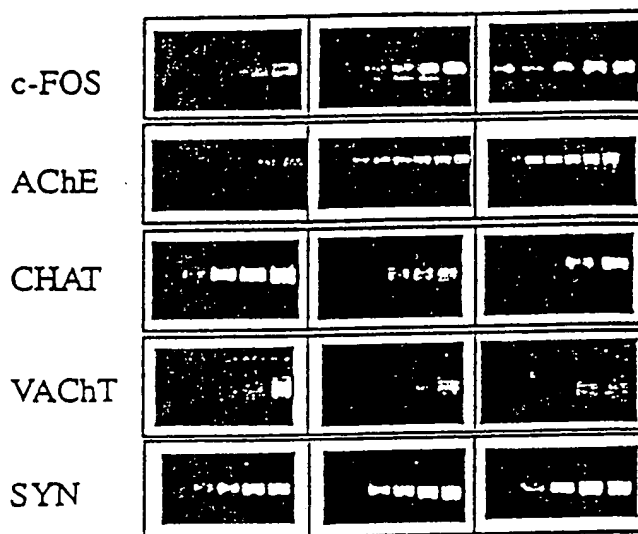
HYDROPHILIC H LLSATV E4 E6 DTLDEAERQWKAEFHRWSSVMVHWNQFDHYSKQDRCSL (SEQ ID NO:5)

PI-LINKED H LLSATV E4 E5 ASEAPSTCPGFTHGEEAPRGLPLPLLHCLLLFLSHLRL (SEQ ID NO:4)

READTHROUGH H LLSATV E4 I4 (SEQ ID NO:2) E5 GMQGPAGSGWEEGSGSPPGVTPLFSP (SEQ ID NO:3)

FIGURE 2

CONTROL stress anti AChE



New III β cycle no. \longrightarrow
 ... | : | ...

B. CONTROL	anti AChE
	



FIGURE 3

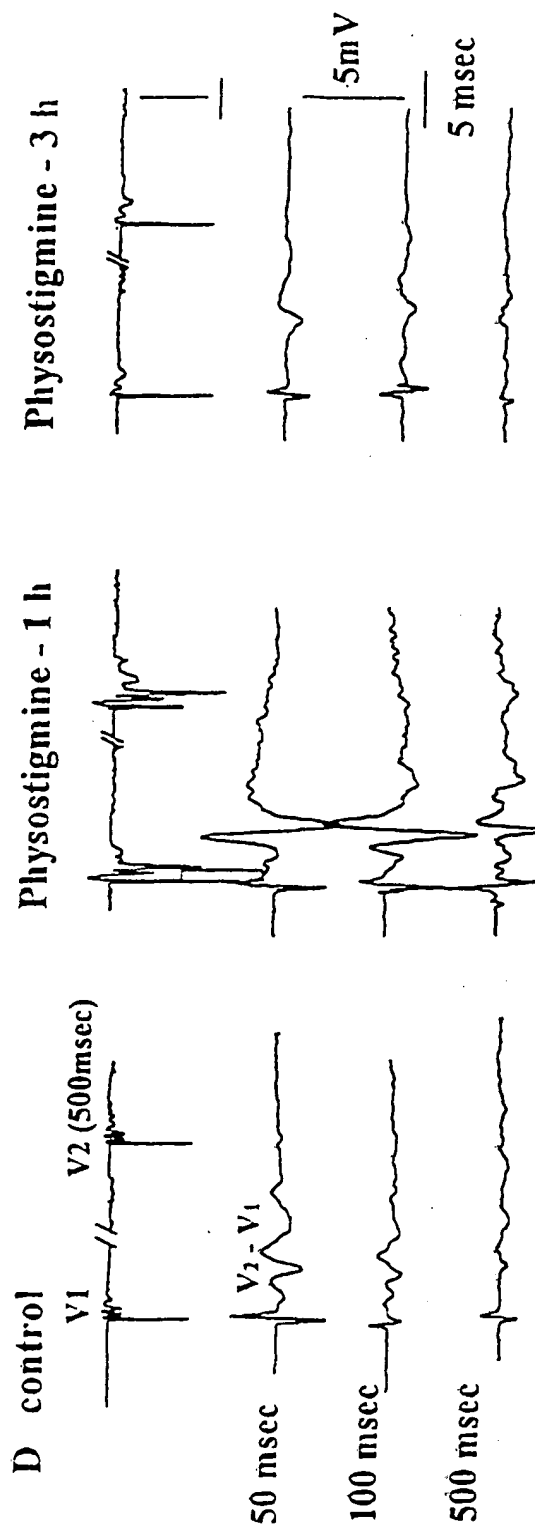
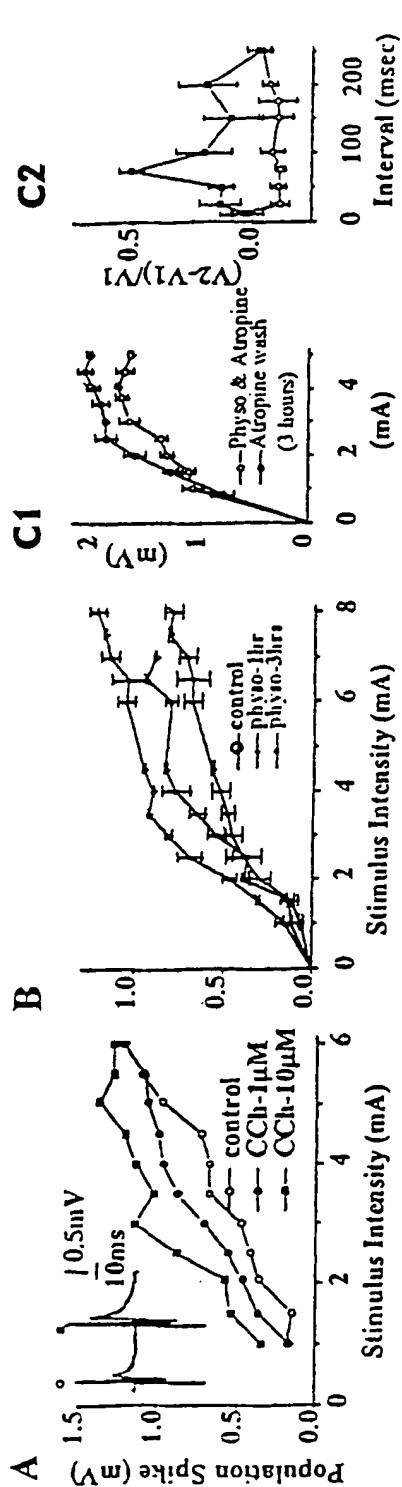


FIGURE 4

BAPTA-AM in medium

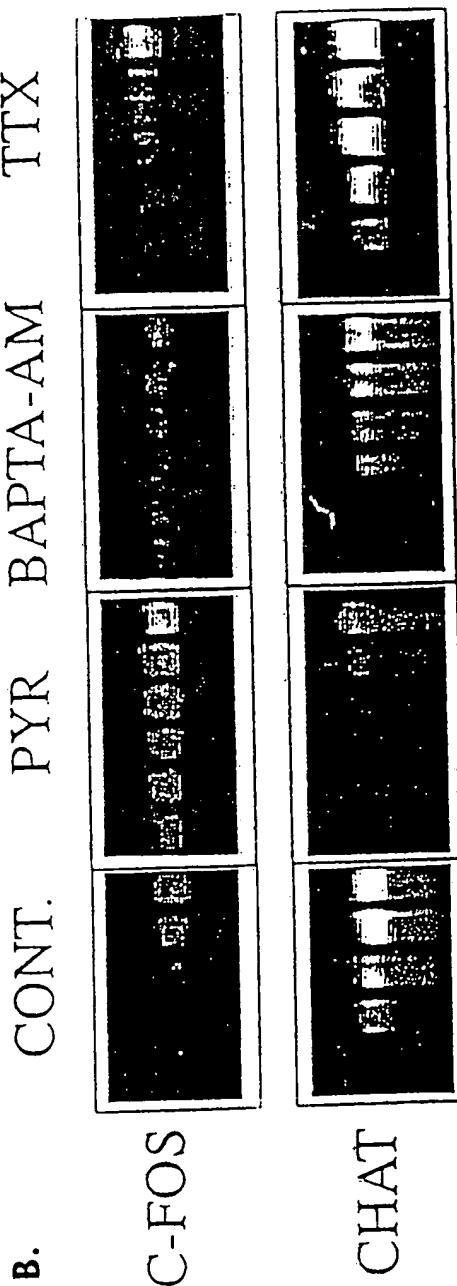
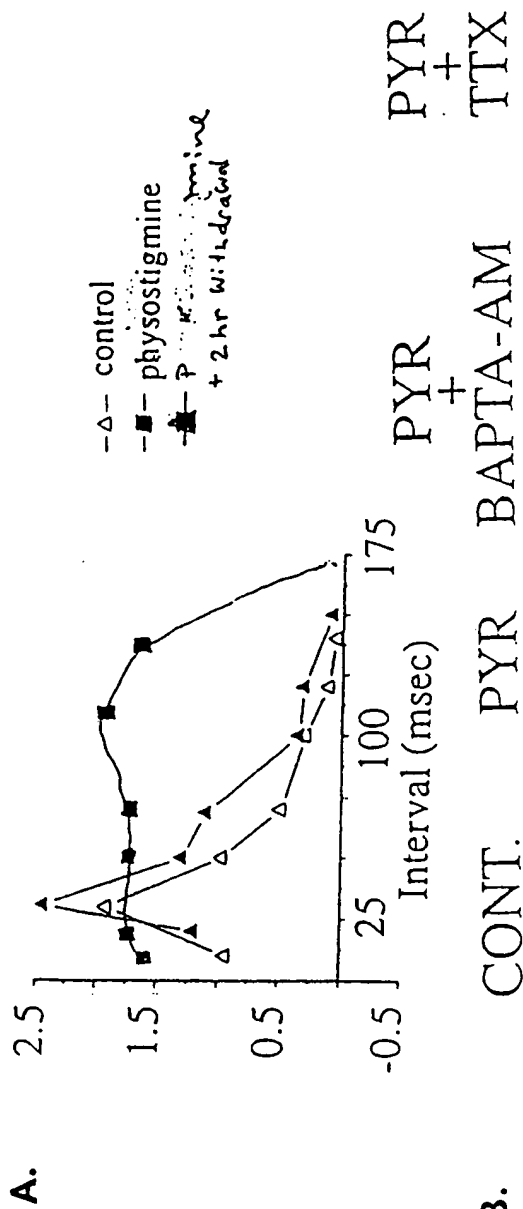


FIGURE 5

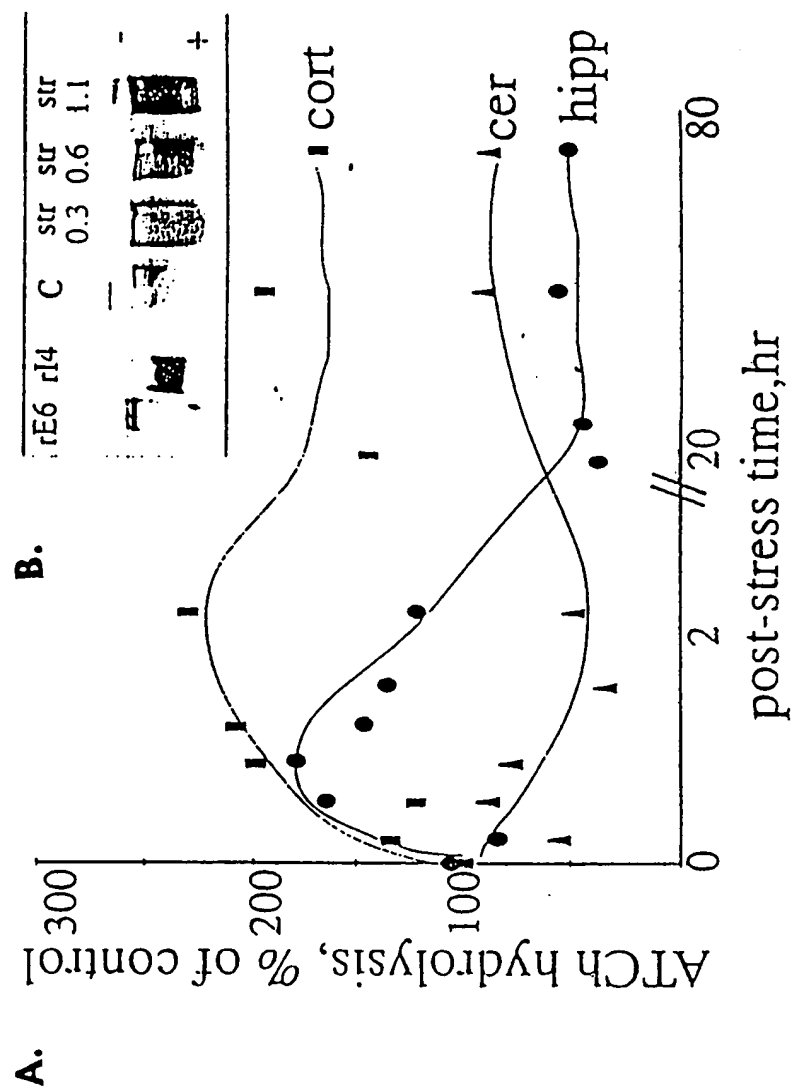
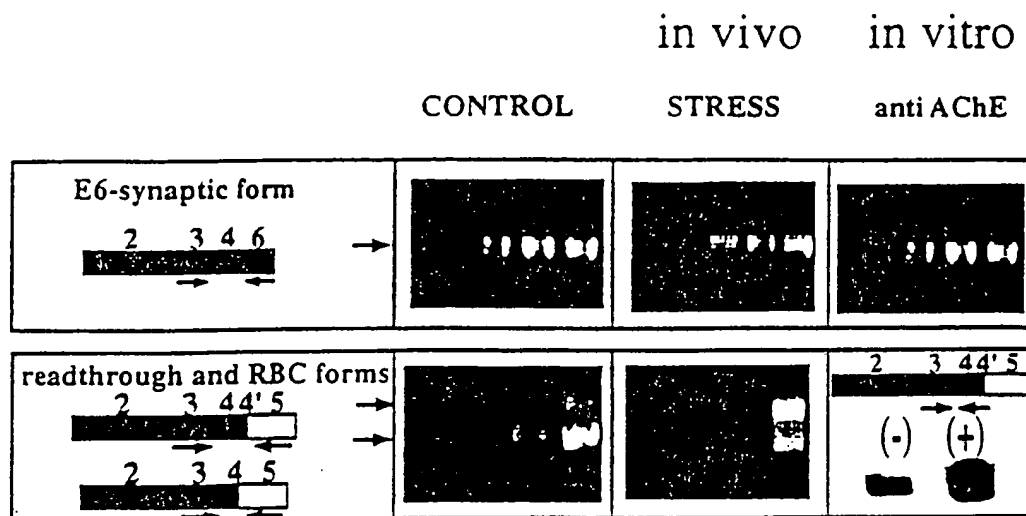


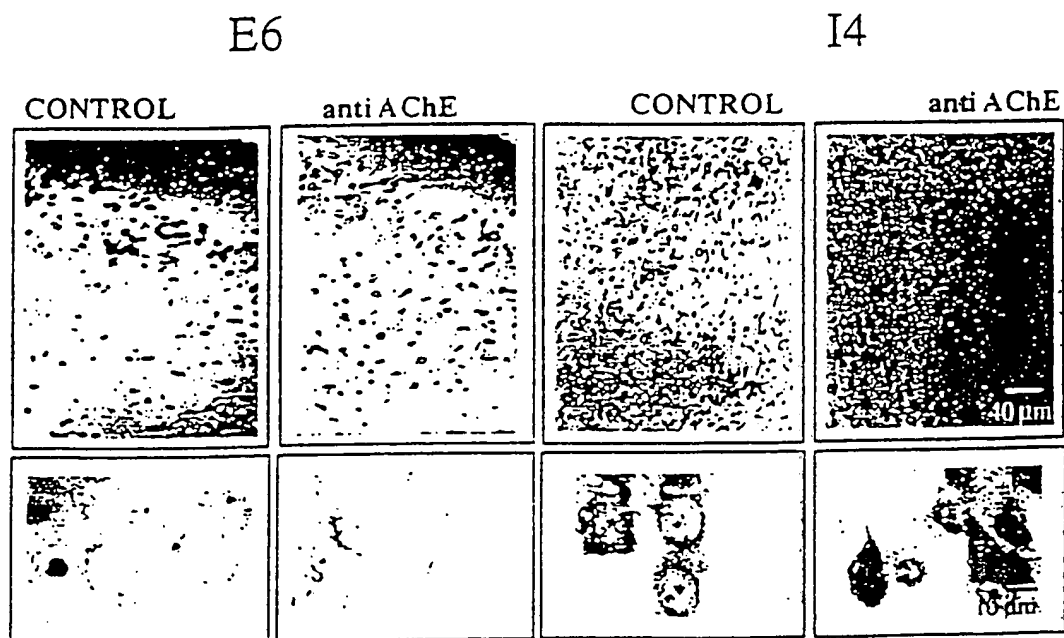
FIGURE 6

6/14

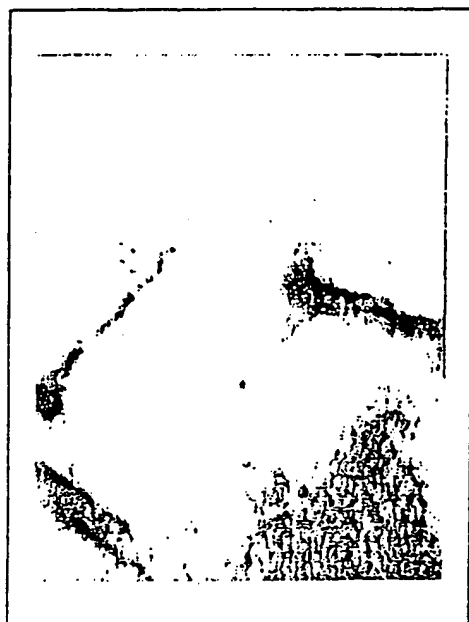
A. ACHE mRNA variants



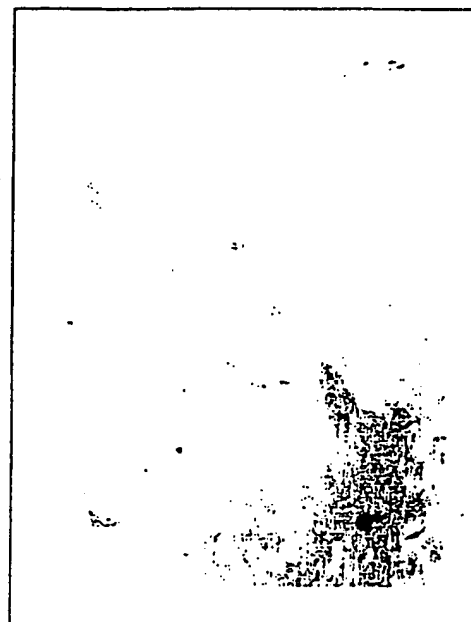
B. AChEmRNA *in situ*



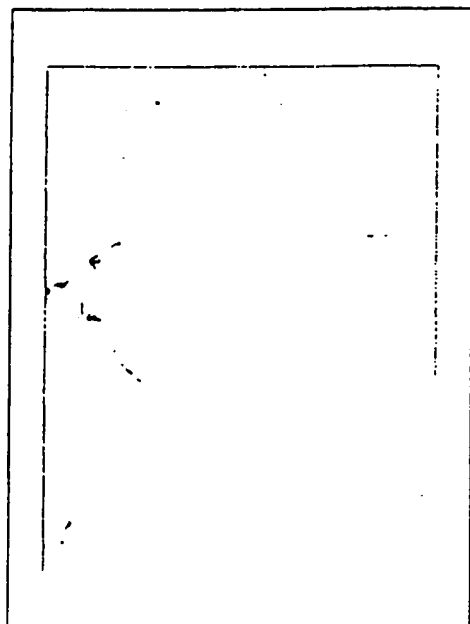
B. stress



D. atropine



A. control



C. adrenaline

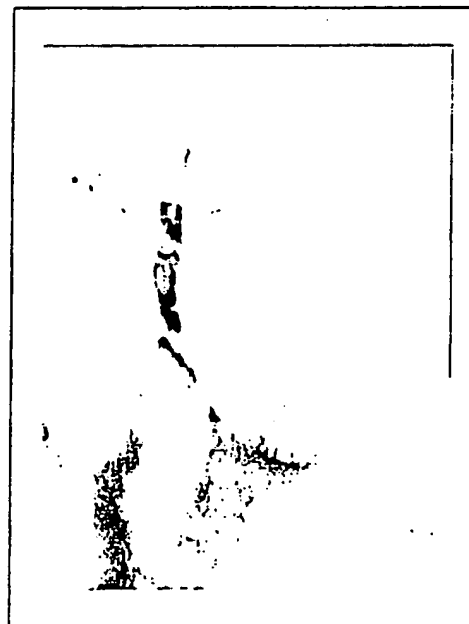


FIGURE 8

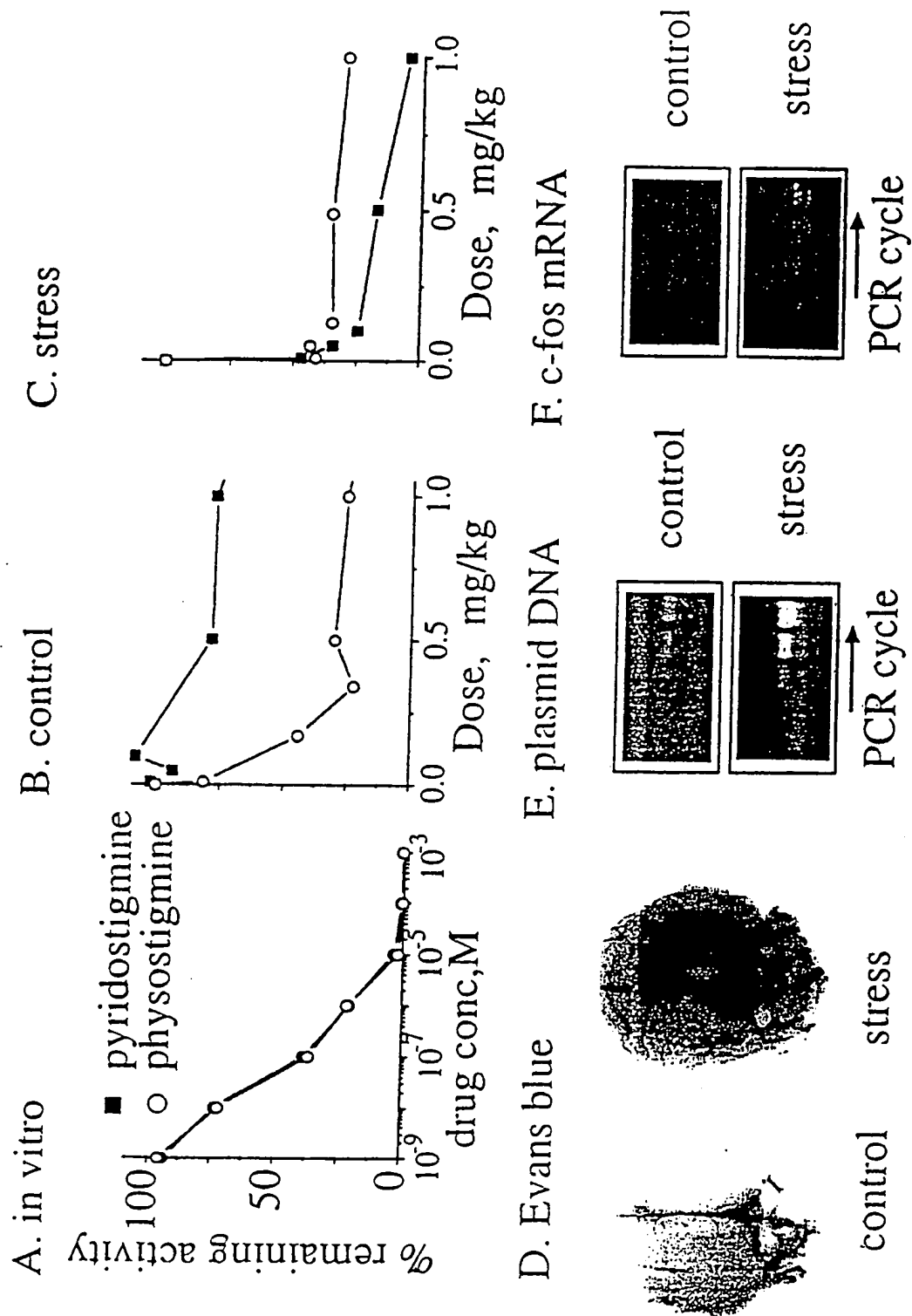
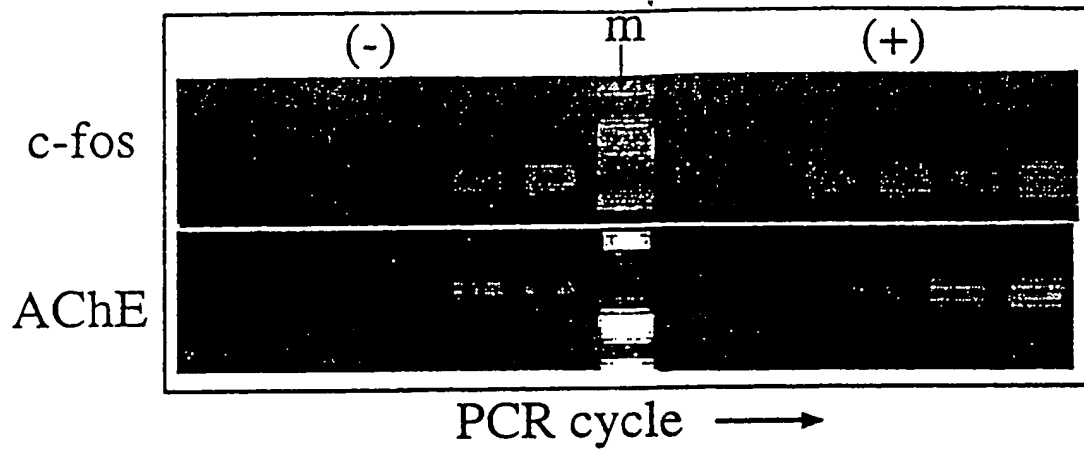


FIGURE 9

A. mRNA, IN VIVO



B. FIELD POTENTIALS, EX VIVO

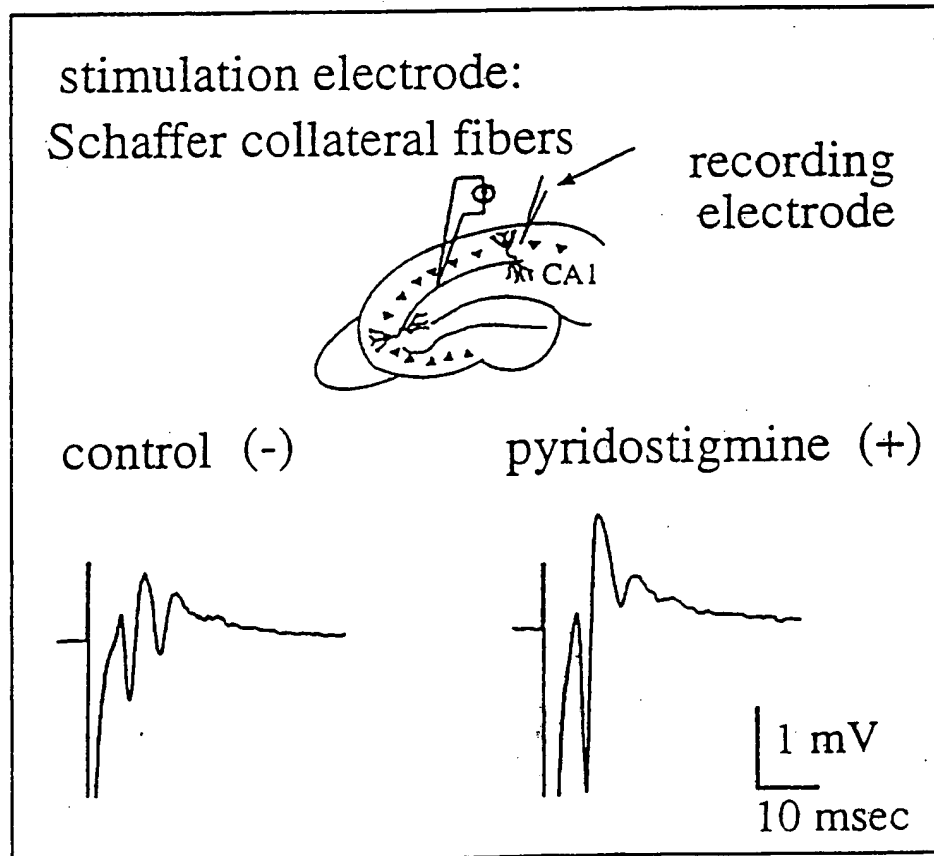


FIGURE 10

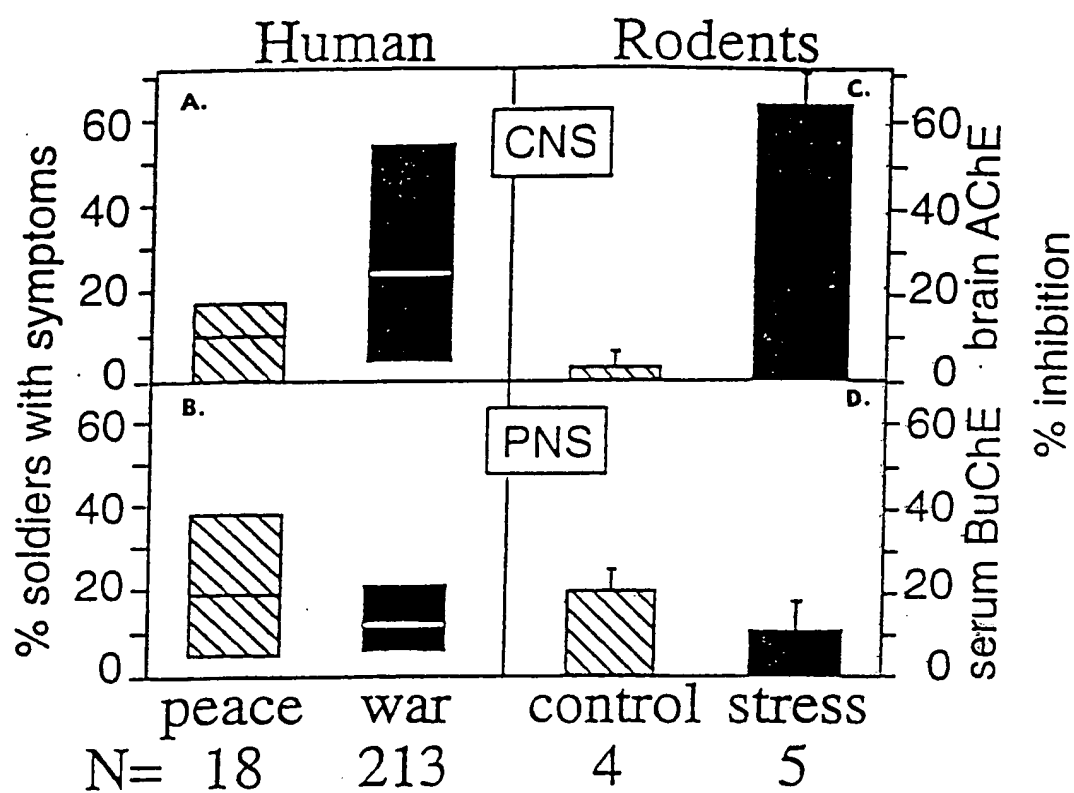


FIGURE 11

control population: normal CTs - Lt

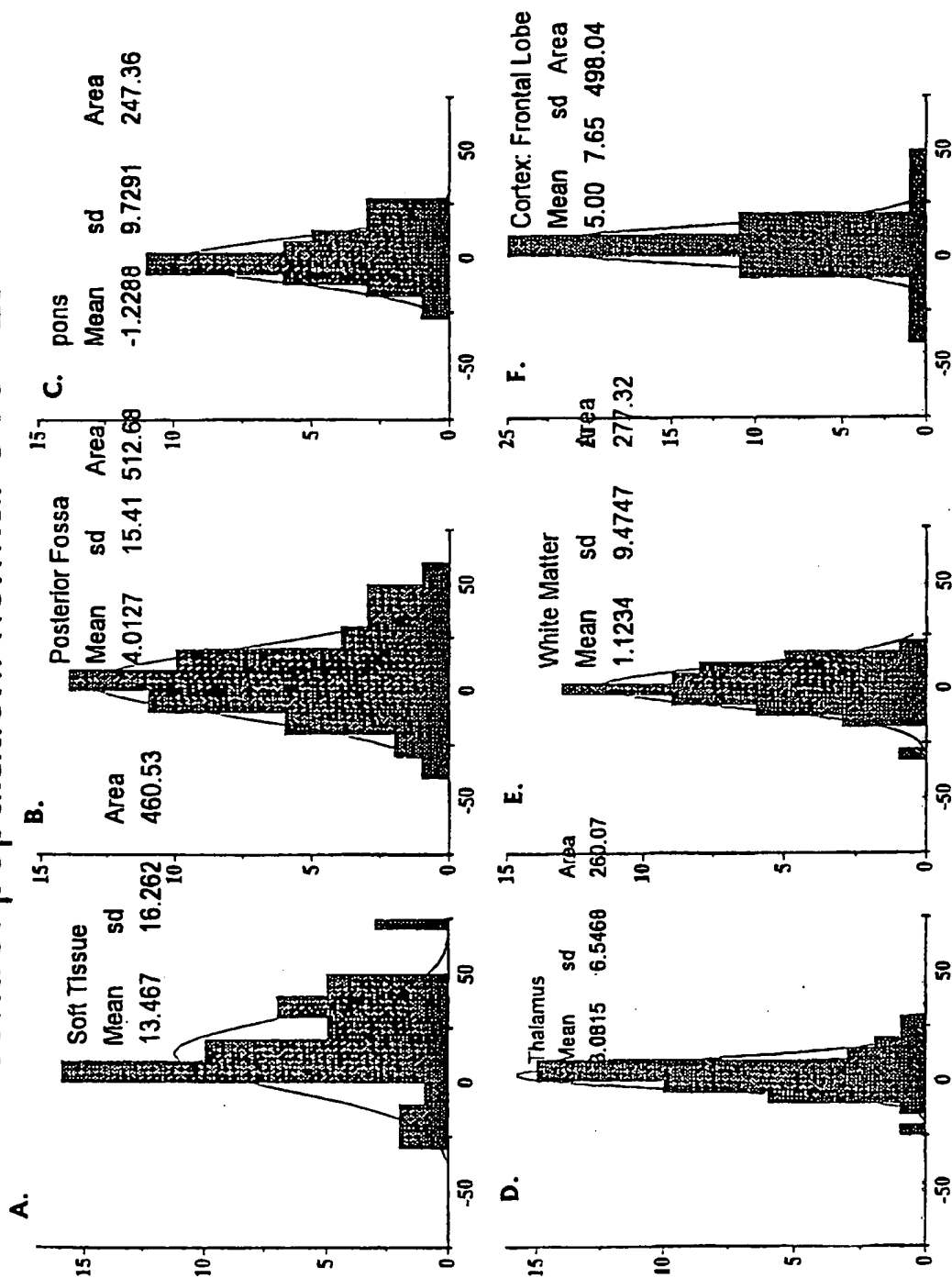


FIGURE 12

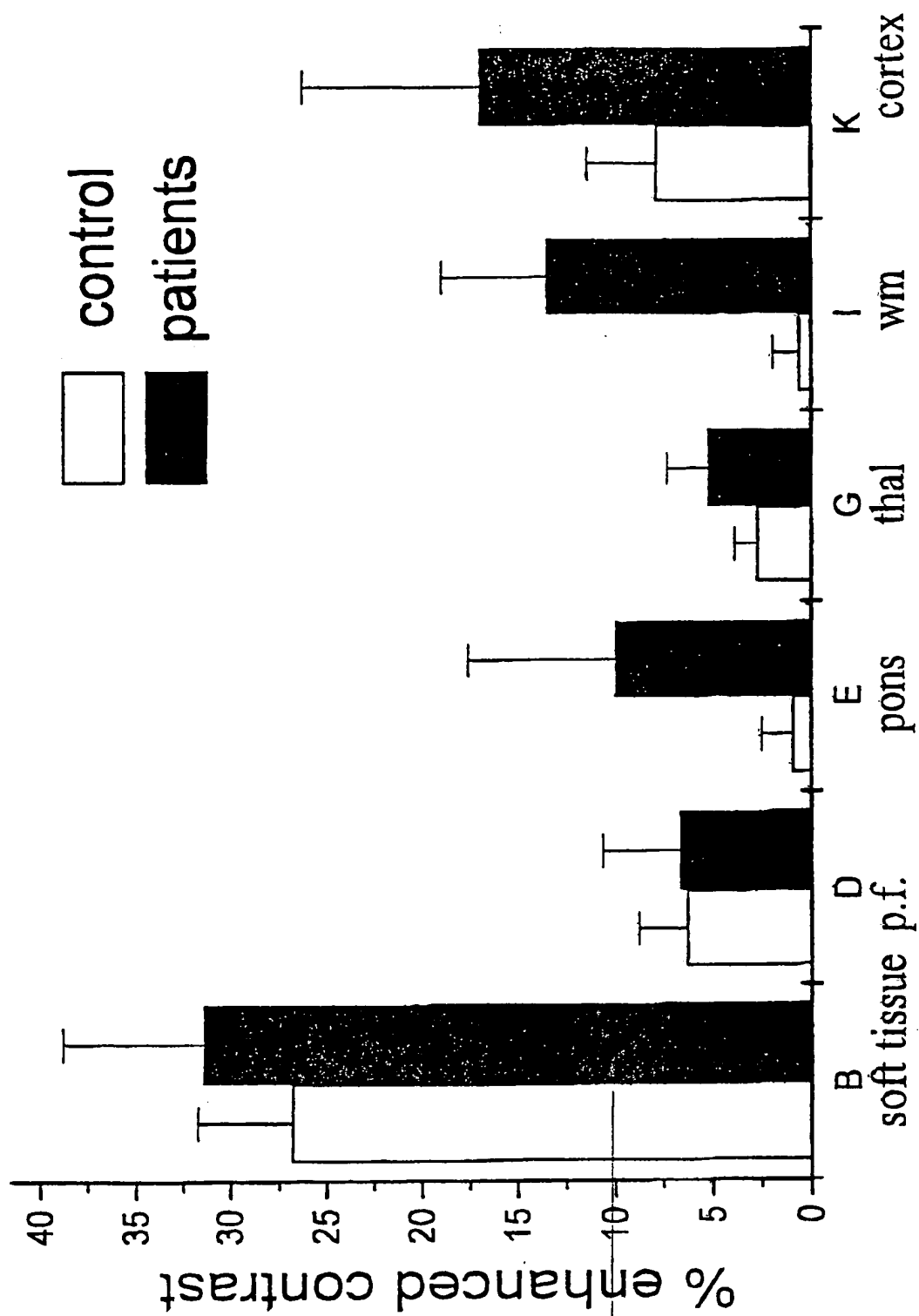


FIGURE 13

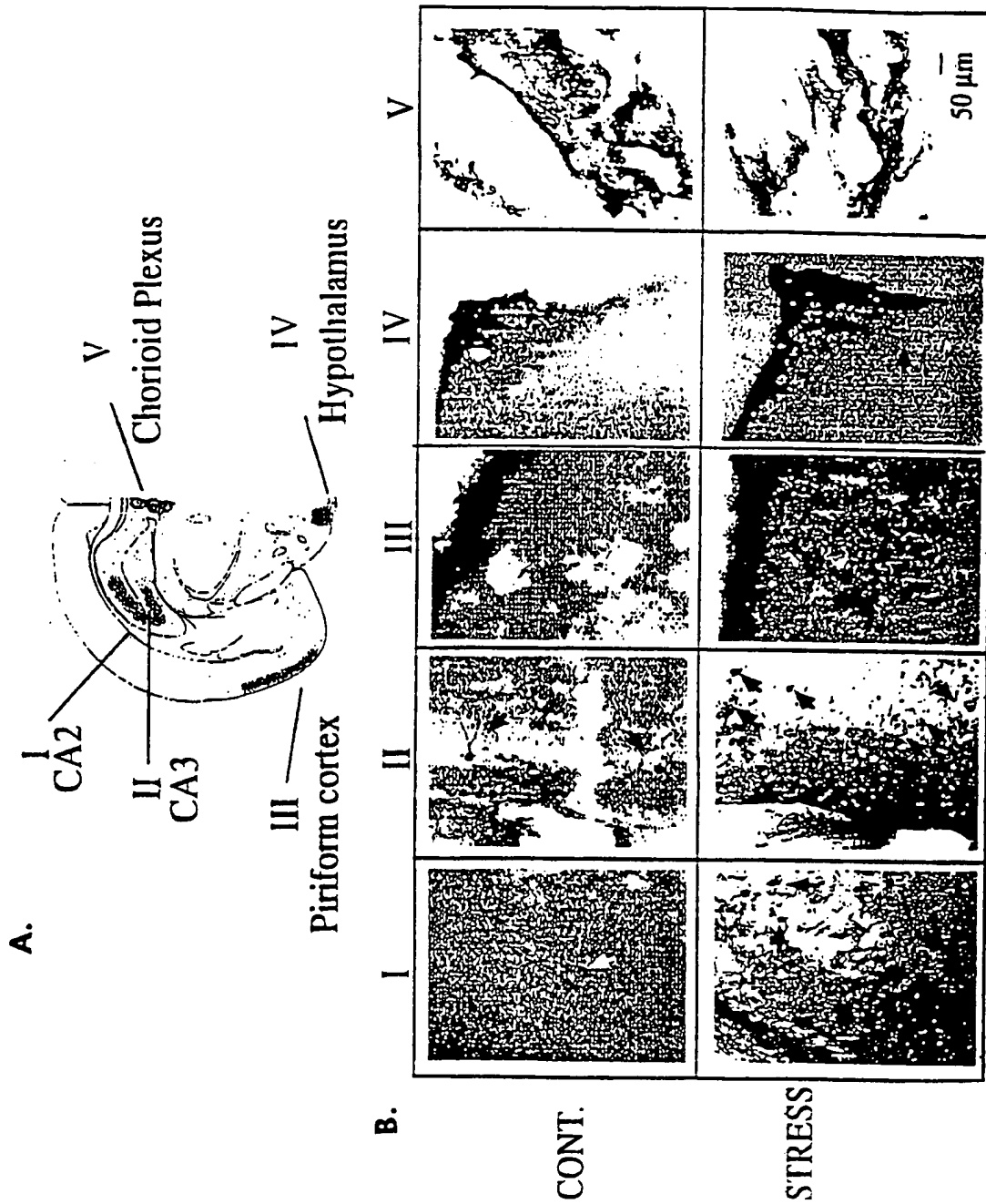


FIGURE 14

Adrenergic Manipulations of BBB Permeability to Pyridostigmine

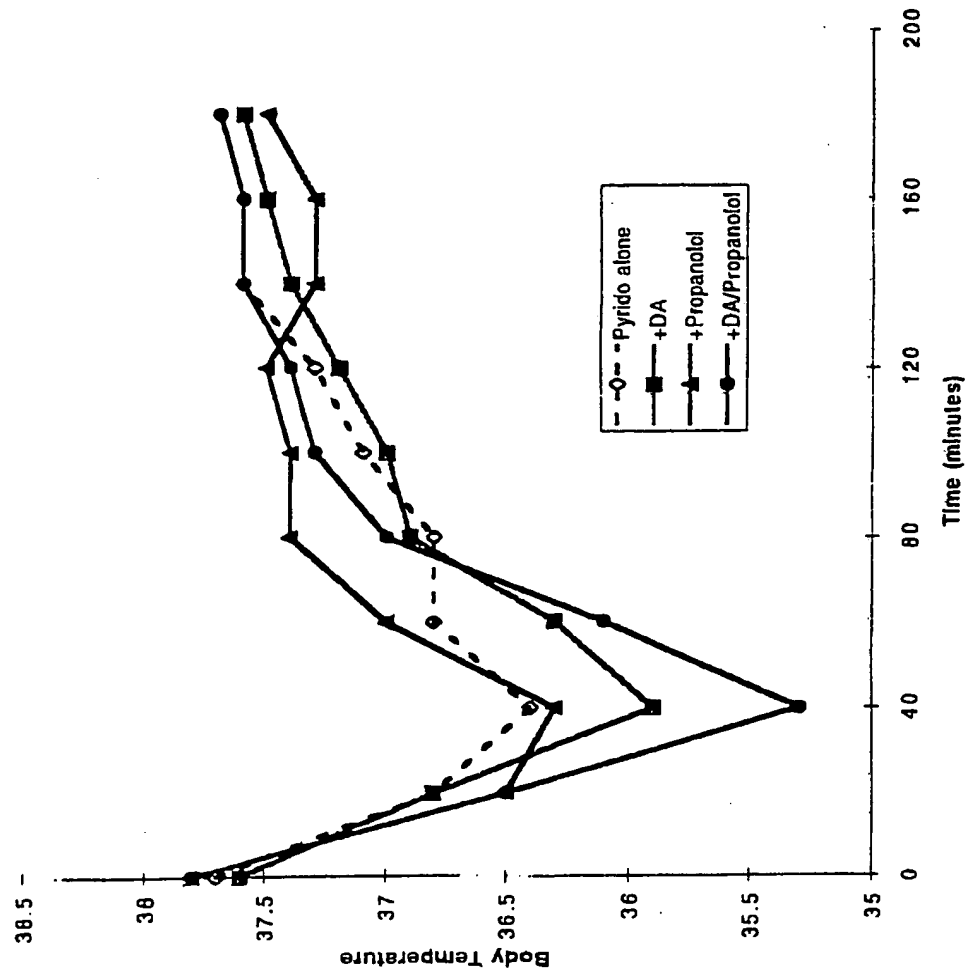


FIGURE 15





INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US97/21696 (22) International Filing Date: 20 November 1997 (20.11.97) (30) Priority Data: 60/031,194 20 November 1996 (20.11.96) US 60/035,266 12 December 1996 (12.12.96) US 60/053,200 21 July 1997 (21.07.97) US (71) Applicant (for all designated States except US): YISSUM RESEARCH DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM [IL/IL]; Jabotinsky Street 46, P.O. Box 4279, 91042 Jerusalem (IL). (71) Applicant (for MW only): KOHN, Kenneth, I. [US/US]; 6761 Alderly Way, West Bloomfield, MI 48322 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): SOREQ, Hermona [IL/IL]; HaMaayan Street_14, 95903 Jerusalem (IL). FRIEDMAN, Alon [IL/IL]; Moshav Maslul, 85112 M. Post HaNeguev (IL). SEIDMAN, Shlomo [IL/IL]; 90909 Neve Daniel (IL). KAUFER, Daniela [IL/IL]; Haguilboa Street_10/6, 90805 Mevasseret Zion (IL).		(74) Agents: KOHN, Kenneth, I. et al.; Kohn & Associates, Suite 410, 30500 Northwestern Highway, Farmington Hills, MI 48334 (US). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: A METHOD AND COMPOSITION FOR ENABLING PASSAGE THROUGH THE BLOOD-BRAIN BARRIER (57) Abstract A pharmaceutical composition for facilitating passage of compounds through the blood-brain barrier comprising the agent ACHE-14 readthrough (SEQ ID No:1) splice variant or the I4 peptide (SEQ ID No:2) and analogues of each thereof and a pharmaceutically acceptable carrier is disclosed. Alternatively, the pharmaceutical composition for facilitating passage of compounds through the blood-brain barrier can comprise the agents adrenaline, atropine, dopamine and/or an adrenergic combination and a pharmaceutically acceptable carrier. The composition can comprise at least two of the agents. The composition of the present invention can optionally include the compound to be transported across the blood-brain barrier. Alternatively, the compound can be co-administered (simultaneously) with the composition or be administered at some point during the biologically effective period of the action of the composition. The present invention provides a method for administering a compound to the CNS of an animal by subjecting the animal to a stress-mimicking agent or treatment. This agent or treatment facilitates disruption of the blood-brain barrier. During the period that the BBB is opened or disrupted a compound can be administered such that the compound is enabled to pass through the disrupted BBB into the CNS.		

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